REMARKS

With entry of this amendment, claims 8-16 and 20-24 are pending. Claims 8-16 are withdrawn from consideration. New claims 20-24 have been entered. Claim 1 has been canceled and rewritten as claim 20. New claims 21-24 recite methods of using the transgenic mouse of the invention. The claims are supported in the specification and the originally filed claims. No new matter has been added. Reconsideration is requested.

INFORMATION DISCLOSURE STATEMENT

The Examiner indicated that the Information Disclosure Statement failed to comply with 37 CRF 1.98(a)(2). It is the Examiner's position that reference 6 appears to be an article in the Japanese language and that a copy has not been provided. This reference, an abstract, was listed in the International Search Report, which was filed concurrently with the application. Copies of the cited references were not provided, as they should have been furnished by WIPO. The Examiner was requested to contact applicant's representative if copies were needed. A copy of this reference (Osamu TAKEUCHI et al., Osaka Kokusai Kaigijo, 3-B-"17-11-O/P December 13, 2001), along with an English translation, is enclosed for the Examiner's convenience. It is respectfully submitted that the reference be considered and made of record.

NONSTATUTORY DOUBLE PATENTING REJECTION

Claims 1, 5 and 6 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 52 of copending Application No. 09/889,324 in view of Thoma-Uszynski et al. The Examiner stated that "Mycobacterium are a species of the bacteria genus. The TLR2 gene recited in claim 52 of '324 is encompassed by the 'gene encoding a protein specifically recognizing mycobacterial lipoproteins/lipopeptides' of claim 1 of the instant application. As such, the claims are drawn to inventions that overlap in scope". To the extent that this rejection may be considered applicable to the presently pending claims, it is traversed for the following reasons.

The present claims are restricted to a transgenic mouse, wherein the genome of the mouse comprises a homozygous inactivation of Toll-like Receptor 1 (TLR1) gene and a method for using such a mouse. It is believed that the scope of these claims does not overlap with claim 52 of the co-pending U.S. patent application No. 09/889,324 (claim 1 of U.S. Patent No. 7078585). Reconsideration and withdrawal of the rejection are respectfully requested.

OBJECTION TO CLAIM 4

Claim 4 was objected to because of a minor informality. Claim 4 has been canceled, thereby obviating this objection. New claims 20-22 refer to TLR 1 as "Toll-like Receptor 1" at the first recitation of such term, as helpfully suggested by the Examiner.

REJECTION UNDER 35 U.S.C. §112

Claims 1-7 were rejected under 35 USC § 112, first paragraph, because the specification is not considered to be enabling for the recited scope. It was the Examiner's position that while the specification is enabling for

(1) a transgenic mouse wherein the genome of said mouse comprises a homozygous inactivation of the TLR1 gene, wherein said TLR1 gene encodes a polypeptide that recognizes triacylated mycobacterial lipoproteins, wherein peritoneal macrophages of said mouse exhibit decreased responsiveness to said triacylated mycobacterial lipoproteins, wherein said peritoneal macrophages also comprise a homozygous disruption of the TLR1 gene;

and

(2) a transgenic mouse wherein the genome of said mouse comprises a homozygous inactivation of the TLR2 gene, wherein said TLR2 gene encodes a polypeptide that recognizes triacylated or diacylated mycobacterial lipoproteins, wherein peritoneal macrophages of said mouse exhibit decreased responsiveness to said mycobacterial lipoproteins, wherein said peritoneal macrophages also comprise a homozygous disruption of the TLR2 gene;

that the specification does not reasonably provide enablement for the scope of the pending claims.

Although Applicants do not agree with the Examiner, in order to advance prosecution, Claim 1 has been rewritten as Claim 20 to recite a transgenic mouse which falls within the criteria indicated by the Examiner as enabled. Claims 2-7 have been canceledIt is believed that Claim 20 is free of the rejection. Reconsideration and withdrawal thereof is respectfully requested.

Claims 1-3, 5 and 6 were rejected under 35 USC § 112, first paragraph, as failing to comply with the written description requirement. It was the Examiner's position that the specification does not describe the claimed subject matter in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time the

invention was filed.

Although applicants disagree, in order to advance prosecution, Claim 1 has been rewritten as claim 20 to narrow the scope of the claim to that which is believed to be acceptable to the Examiner. Claims 2, 3, 5, and 6 have been canceled. Reconsideration and withdrawal thereof is respectfully requested.

REJECTION UNDER 35 U.S.C. §112, second paragraph

Claims 1-7 were rejected under 35 USC § 112, second paragraph, as being indefinite. Claim 1 has been rewritten as claim 20, and is believed to be free of the rejection. Claims 2-7 have been canceled. Reconsideration and withdrawal of the rejection are respectfully requested.

REJECTION UNDER 35 U.S.C. §102(a)

Claims 1-7 were rejected under 35 USC § 102(a) as being anticipated by Takeuchi et al. Claims 1-7 were rejected under 35 USC § 102(a) as being anticipated by Alexopoulou et al, as evidenced by Takeuchi et al. To the extent that these rejections may be considered applicable to the presently pending claims, they are traversed for the following reasons.

Filed herewith is a certified English translation of the foreign priority document. It is believed that the rejections based on Takeuchi and Alexopoulou references will be overcome by the submission of this document. Reconsideration and withdrawal of the rejections are respectfully requested.

Claims 1-7 were rejected under 35 USC § 102(a) as being anticipated by Henneke et al. To the extent that this rejection may be considered applicable to the presently pending claims, it is traversed for the following reasons.

Henneke et al. only describes that macrophages of TLR1 knockout mice responded normally to a heat-labile soluble factor extracted from group B streptococcus, which is different from lipoprotein. The Henneke reference does not disclose a method for using TLR1 knockout mice as model mice non-responsive to triacylated micobacterial lipoprotein or synthetic triacylated lipopeptide nor that TLR1 specifically recognizes synthetic triacylated lipopeptide, as disclosed by the present invention. It is believed that the presently claimed invention is not disclosed or suggested by Henneke et al. Reconsideration and withdrawal of the rejection are respectfully requested.

REJECTION UNDER 35 U.S.C. §102(b)

Claims 1-7 were rejected under 35 USC § 102(a) as being anticipated by Thoma-Uszynski et al. as evidenced by Takeuchi et al. To the extent that this rejection may be considered applicable to the presently pending claims, it is traversed for the following reasons. The Examiner stated that Thoma-Uszynski et al. teaches a TLR2 knockout mouse with impaired macrophage reactivity toward the 19-kD mycobacterial lipoprotein. In order to advance prosecution, the claims have been restricted to a transgenic mouse and uses, wherein the genome of the mouse comprises a homozygous inactivation of the Toll-like Receptor 1 (TLR1) gene; wherein the TLR1 gene encodes a polypeptide that recognizes triacylated mycobacterial lipoproteins; wherein peritoneal macrophages of the mouse exhibit decreased responsiveness to the triacylated mycobacterial lipoproteins; and wherein the peritoneal macrophages also comprise a homozygous disruption of the TLR1 gene. It is respectfully submitted that the presently pending claims are not anticipated by Thoma-Uszynski et al. Reconsideration and withdrawal of the rejection are respectfully requested.

All objections and rejections having been addressed, it is respectfully submitted that this application is in condition for allowance, and Notice to that effect is respectfully requested.

Respectfully submitted,

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3-B-W17-11-O/P

Analysis of mechanism recognizing bacteria components such as TLR2

Toll-like receptor (TLR) family plays an important role to recognize pathogens through innate immunity. 10 TLRs have been reported so far, and among these, TLR4 and TLR9 are essential for the recognition of LPS and bacterial DNA, respectively. Last year, the present inventors have generated TLR2- and TLR6-knockout (KO) mice, and reported that TLR2 is essential for recognition of bacterial lipoprotein, peptidoglycan, or microplasmal lipoprotein (MALP), and that TLR6 KO mice do not respond to MALP. This time, to further analyze in detail the MALP recognizing-mechanism by TLR2/TLR6, TLR2 and TLR6 were reconstituted by using TLR2/TLR6 KO fibroblasts (EF) with a retroviral vector. By co-expressing TLR2 and TLR6 in TLR2/TLR6 KO EF, the response to MALP was obtained, while it was not obtained separately. Interestingly, the response to MALP was recovered by co-expressing chimeric constructs wherein intracellular regions of TLR6 are linked in the extracellular region of TLR2 and the reverse chimeric construct. Therefore, it was assumed that both extracellular and intracellular regions of TLR2/TLR6 were necessary for the recognition of MALP and for the activation of signaling pathway. Moreover, by searching the pathogen components recognized in the TLR6/TLR2 system, it was revealed that Glycosylphosphatidylinositol (GPI) anchor derived from Trypanosoma cruizi is also recognized by TLR2 and TLR6. At the moment, investigations are made for recognition system of bacterial components such as bacterial lipoprotein, which necessitates TLR2 but independent of TLR6.

necessitates TLR2 but independent of TLR6.

3-B-W17-9-P ニワトリ Toll-like receptor(TLR) の単離と機能解析：福井理[1,2]、松本美佐子[1]、瀬谷司[1] ([1]大阪成人病セ・免疫、[2]奈良先端大・バイオ)

Toll-like receptor(TLR) は、ショウジョウバエにおけるパターン認識レセプター Toll のホモログとして報告された。哺乳類で報告されている数種の TLR のうち TLR2 と 4 は、Toll と同様に外来異物の認識に関わることが示されている。しかし、Toll/TLR の機能が生物の進化と共にどのように変化してきたのかは不明である。そこで、哺乳類以外の脊椎動物で TLR の単離および機能解析を行い、哺乳類のそれと比較することを試みた。
まず、ニワトリのファブリキウス嚢 cDNA ライブラリーを鋳型とし、縮重プライマーを用いて PCR 法により 2 種のニワトリ TLR(type1、type2) の cDNA をクローニングした。type1 と type2 は、異なる遺伝子から構成されているが、開始メチオニン付近と LRR ドメインの一部以外のアミノ酸配列は全く同じであり、共に一次構造上ヒト TLR2 に最も高い相同性を示した。ニワトリ TLR 両タイプの mRNA およびタンパク質の発現分布を調べたところ、広範な組織で発現が認められた。特に、結合組織においてそのタンパク質の発現が多かった。ニワトリ TLR のパターン認識レセプターとしての機能をヒト腎由来 293 細胞を用い、NF-κB の活性化を指標に解析した結果、*Mycoplasma fermentans* 由来のリポペプチド MALP-2 をリガンドとしたときに、両タイプの発現細胞で応答が認められた。一方、*E.coli* または *S.typhimurium* 由来の LPS をリガンドとしたときは、type2 発現細胞でのみ応答が認められ、特にヒト CD14 を共発現させた場合に応答が顕著であった。
以上の結果より、鳥類では遺伝子の重複によりできた複数の TLR がパターン認識レセプターとして機能しており、そのシステムは哺乳類のそれとは異なる可能性が示唆された。

0873

3-B-W17-10-O/P マウス樹状細胞における Toll-like receptor を介した遺伝子発現機構の解析：星野克明[1]、岩部富夫[1,2,3]、竹内理[1,2]、改正恒康[1,2]、審良静男[1,2] ([1]大阪大・微研・癌抑制遺伝子、[2]科学技術振興事業団、[3]鳥取大・医・産婦人科)

【目的】昨年の学会では、TLR4 を介したシグナル伝達経路に、IRF3 を用いる MyD88 非依存性経路があり、この経路によりインターフェロン誘導性遺伝子が発現することを報告した。今回、我々は MyD88 非依存性経路の存在しない TLR9 の刺激を行った結果、TLR4 の場合と同様にインターフェロン誘導性遺伝子の発現が見られたので、その遺伝子発現パターンについて解析した。
【方法と結果】野生型マウスおよび各種遺伝子欠損マウス (MyD88-KO マウス、IFN-α／β receptor-KO マウス) の骨髄細胞を、GM-CSF 含有培地で培養することにより、未熟な骨髄由来樹状細胞として用いた。LPS と CpG DNA を用いて、TLR4 および TLR9 の刺激を行い、樹状細胞に発現する遺伝子をノーザンブロット法で調べた。その結果、TLR4 の刺激により、ごく初期に IFN-β の発現が誘導されたが、TLR9 の刺激では IFN-β の発現は時間的に遅延した。また、ケモカイン IP-10 は、TLR4 刺激により直接的に発現誘導され、同時に細胞自身が分泌した IFN-β により 2 次的に発現が増強された。TLR9 刺激では、直接的な IP-10 の発現誘導は見られず、2 次的に発現誘導されるのみであった。他の遺伝子についても検討中である。
【考察】刺激を行う TLR の種類により発現する遺伝子のパターンが異なることから、TLR が誘起するシグナル伝達系路にそれぞれ違いがあると考えられる。病原微生物の種類に応じ自然免疫反応のパターンを変えて、適切な生体防御反応を誘導していると考える。

3-B-W17-11-O/P TLR2 を中心とした菌体成分認識機構の解析：竹内理[1,2]、堀内亮郎[1,2]、審良静男[1,2] ([1]阪大・微研・癌抑制、[2]科技団)

Toll-like receptor(TLR) ファミリーは自然免疫による病原体認識に大きな役割を果たしている。現在までに 10 種類の TLR が報告され、そのうち、TLR4 は LPS、TLR9 は細菌由来 DNA の認識に必須である。昨年我々は TLR2 及び TLR6 のノックアウト (KO) マウスを作製し、TLR2 が細菌由来リポプロテインやペプチドグリカン、マイコプラズマ由来リポプロテイン (MALP) の認識に必須であること、TLR6 KO マウスが MALP に対し反応しないことを報告した。今回、更に TLR2/TLR6 による MALP 認識機構を詳細に解析する目的で、TLR2/TLR6 KO 線維芽細胞 (EF) を用いてレトロウイルスベクターにより TLR2、TLR6 の再構成を行った。TLR2/TLR6 KO EF に TLR2、TLR6 を共発現させることで MALP に対する反応性が獲得されたが、それぞれ単独では獲得されなかった。興味深いことに TLR2 の細胞外領域に TLR6 の細胞内領域をつなげたキメラ、及びその逆のキメラを共発現させることによっても MALP に対する反応性が回復した。従って、MALP の認識及びシグナル伝達系路の活性化の両方に TLR2/TLR6 の細胞外及び細胞内領域が必要であると考えられた。また、さらに TLR6/TLR2 システムで認識される病原体成分の検索を行ったところ Trypanosoma cruzi 由来 Glycosylphosphatidylinositol(GPI)anchor も TLR2 及び TLR6 により認識されることが明らかとなった。現在、細菌由来リポプロテインなど TLR2 を必要とするが TLR6 に依存しない菌体成分の認識機構に関しても検討を加えている。

0875

3-B-W17-12-P 各種 DNA による細胞活性化における TLR9 の役割の検討：邊見弘明[1,2]、改正恒康[1,2]、竹内理[1,2]、星野克明[1,2]、竹田潔[1,2]、審良静男[1,2] ([1]阪大・微研・癌抑制、[2]科技団)

CpG モチーフを含む DNA(CpG DNA) は、マクロファージや樹状細胞、B 細胞、NK 細胞を活性化することが知られている。われわれは、様々な菌体構成成分の認識に関わる Toll-like receptor(TLR) ファミリーのうち TLR 9 がこの CpG DNA による細胞の活性に必須であることを報告した。しかし、この CpG モチーフには、様々な配列が知られている。そこで、われわれは、TLR9 ノックアウトマウスを用いて様々な配列をもつ CpG DNA への反応性を検討した。CpG モチーフの配列が 5'-GACGTT-3'、5'-GTCGTT-3'、5'-AACGTT-3' をそれぞれ含む合成オリゴ DNA への反応性をマクロファージからの TNF-α の産生や脾細胞の増殖を指標にして検討したところ、TLR9 欠損マウス由来細胞は、これら 3 種の CpG DNA へ反応しなかった。さらに、*E. coli* 由来ゲノム DNA による脾細胞の増殖を検討したところ、他の CpG DNA と同様に TLR9 欠損マウス由来細胞は増殖を示さなかった。このように、TLR9 は、合成オリゴ DNA のみならず細菌由来 DNA の反応にも必須であり、また、CpG モチーフの細かい配列の違いに関わらず CpG DNA の認識に関与していることが判明した。さらに、われわれは、ポリグアノシン DNA や GC リッチな配列を有す GR1 による脾細胞や骨髄細胞の増殖など CpG DNA モチーフを含まない DNA による細胞の反応が TLR9 を介するかどうかも検討を行ったので、その結果についても紹介したい。